A Review of Cancer Vaccine
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Abstract

In 2012 cancer disease was the second leading cause of death in United States. The major limitations of the conventional cancer therapy is the lack of specificity, collateral damage to normal tissue, which in some case is worse than the abnormalities in cancer cells, and the rates of cure of these conventional therapies are still low. Cancer vaccines, a new method propose to destroy and prevent cancer development, represent an incomparable and unique approach to treating cancer. The greatest advantage of this approach is the ability to target both surface and intracellular antigens through the activation of the cellular immune response and humoral immune response, as well as the greater duration response, which avoid the need of long-term and multiple immune treatments. Moreover, this approach activates the immunologic memory response, which could potentially represent the greatest advantage of the immune-based therapy because it can establish a durable therapeutic effect independent of repetitive cycles of therapy as the traditional method require. Although, to date there are a couple of cancer vaccines approved by the US Food and Drug Administration and a numbers of vaccines undergoing preclinical and clinical trial which are hold promising to be an effective anticancer therapy. The scope of this review attain to defined cancer vaccines disease, the immune system response against tumor cells, approaches for the development of cancer vaccines that are in clinical trials as well as different considerations that are taking into consideration for enhancing the efficacy and immunogenicity cancer vaccines.

Introduction

Based on incident data from the National Cancer Institute, the Centers for Disease Control and Prevention, as well as the North American Association of Central Cancer Registries and mortality data from the National Center of Health Statistics, a total of 1,638,910 new cancers were diagnosed and 577,190 deaths from cancer were reported in the United States in 2012. The two leading causes of death was heart disease and cancer together accounted for nearly 50% of all deaths[1].

The scientific communities are making efforts to eradicate this catastrophic illness efficiently, causing the least possible damage to non-cancerous tissue. Currently, the methods used to fight this disease are surgery, radiation, chemotherapy, and endocrine manipulation. These have resulted in substantial improvements in the morbidity and mortality associated with cancer. However, this improvement can be considered only in developing countries which have more access to optimized cancer therapies[2] and according to Jones S. argues improvements of the traditional methods to overcome cancer are unlikely due to mechanisms of therapeutic resistance of the transformed cancer cell[3].
Cancer has been treated with a number of different methods which includes surgery and radiation therapy for local control. Meanwhile cytotoxic chemotherapy and endocrine manipulation have been used to try to control micro-metastatic disease and the disease relapse. Usually, these therapies are typically combined in a specific order to achieve the best control of disease. However, no matter the therapies’ order there are still too many drawbacks that have to be overcome in order to develop an effective therapy treatment. It is known that radiation, chemotherapy, and endocrine therapy alter cell biology in both cancerous cells and normal tissue. This lack of specificity is the major limitation of the true efficacy of conventional cancer therapies. In many case the collateral damage to normal tissue is worse than the abnormalities in cancer cells. In addition, it is considered that the rates of cure of these conventional therapies are still too low[4]. Cancer relapse is usually due to the outgrowth of tumor cell clones that inherited resistance to these conventional therapies. Thereby it can be said that the second major limitation to the efficacy of the conventional standard therapy of cancer is the intrinsic drug resistance. These two specifics barriers provide the motivation to develop an innovative cancer treatment strategy, which can potentially be more specific to transformed cancer cells and with a treatment capacity, that evolve in parallel with the abnormal cells. As expected, different approaches have been proposed. In this review we will focus on those cancer therapy approaches in which are based on vaccine engineering.

Traditional vaccines had have proven to be successful preventing infectious diseases. However, most cancers are not to caused by infectious agents, but rather, by defects in cellular gene expression protein [5]. These proteins are very similar to those found in normal cells, which makes it very difficult to develop vaccines targeting the cancer cells while avoiding the attack of the normal cells at the same time. That is the principal reason why most cancer vaccines are more likely to be useful for treating cancer in patients already with the illness and not for preventing cancer [5].

Cancer vaccines represent an incomparable and unique approach to treating cancer because of their ability to alter the interaction between the host cell immune system cells and the cancer cell. In this approach, the patient immune system is trained to recognize, attack, and destroy cancer cells. There is no evidence of possible drug resistance because this treatment is based on the activation of signaling pathways within the host cell and a high degree of specificity of the response is achieved [6]. Thus it is possible to minimize the likelihood of undesirable side effects of traditional cancer treatment. Moreover, this approach activates the immunologic memory response, which could potentially represent the greatest advantage of the immune-based therapy because it can establish a durable therapeutic effect independent of repetitive cycles of therapy as the traditional method required [5].

Cancer vaccines promise to be a good approach to treating cancer, but do not show to have the same efficiency preventing its formation. These differences have been considered related to multiple factors. First, the immune response for an infection and for abnormal growth cells is quite different. Humoral immunity, which is known as the antibody response control, eradicates acute infections, while cellular immunity, T-cell response, is responsible for the eradication of established cancers and chronically infected cells. Furthermore, the burden of infected or abnormal cancer cells frequently exceeds the immune system cells regulation, thereby limiting the immune response for this chronic disease[6].
It is true that there has been significant progress in developing cancer vaccine, in the last decade. However the concept of stimulating the immune system to fight cancer cells is more than century old. In 1893 William Coley observed the regression of soft-tissue sarcoma in patients with acute Streptococcal bacterial infection[7]. Based on his observation, he tested his patients with intravenous administration of this specific bacterial extract to stimulate tumor-specific immune response[7]. Unfortunately, there was also evidence that his approach was correlated to tumor regression, and this nonspecific activation of the immune response was associated with serious side effects. However, the progress achieved in understanding the molecular and cellular basis of the immunity opened a new wide window for the use of cancer vaccine therapy, as a method to combat developed cancer cells and prevents its development. The mechanisms by which the immune system response is initiated as well as controlled are known in detail[8]. As a result, cancer vaccine, became a potential treatment.

To date, there are already a couple of cancer vaccines approved by the US Food and Drug Administration (US FDA). In 2006, the US FDA approved Gardasil®, the first vaccine specifically to prevent cervical cancer from the human papillomaviruses (HPV) type 6, 11, 16, and, 18[9]. Later on, in 2010, the FDA approved Provenge® a therapeutic cancer vaccine to treat men with advanced prostate cancer. It does not help to get rid of cancer completely but prolongs the survival of the patient[10].

Scientists agree with the fact that immune system cells have a crucial role in treating and preventing cancer development[11]. Thus, cancer vaccine therapy is not a future approach to treat tumor illness; it is an approach that we are already able to develop with great efficacy and immunogenicity with existing technology. This review begins with the introduction of cancer disease, the tumor immune system response, and the identity and role of each cell involved in recognizing and fighting cancer cells. The next section attempts to explain each different approach for the development of cancer vaccines that are in clinical trials as well as different considerations that are taking into consideration enhancing the efficacy and immunogenicity cancer vaccines.

What is Cancer?

Cancer may originate from both genetic disorders and environmental factors. It is a disease in which the cells become abnormal and divide without control, with no hayflick limit as well as no contact inhibition. These cells can invade nearby tissue and they may spread through the bloodstream and lymphatic system[12]. If the spread of these cells is not controlled, cancer cells can turn into tumor mass and could result in death of the organisms.

It is known that the best form of cancer prevention is the early detection of abnormal growth in cells, but with the today’s technology that is not a realistic alternative. So, several techniques have been developed to treat cancer. These techniques include the following:

*Surgery* is the oldest and most widely used treatment against cancer for patients with an early detection. If the abnormal growth is found in an early stage there is a good probability of removing the entire tumor before it spreads out through the body (metastasis). Surgery is seldom
used as a stand-alone treatment. This treatment tends to be combined with radiation therapy and/or chemotherapy[12].

Radiation therapy use localized high voltage energy radiation to a specific part of the body containing cancerous growth to attack the reproduction of cancer cells, to decrease a tumor so that it can further be removed through surgery, or to prevent tumor growth following surgery¹. A drawbacks of this treatment is that it affects normal cells along with the cancer cells which can lead to several ungrateful side effects, such as fatigue, dryness, nausea and vomiting, among other[12].

Chemotherapy is a treatment of cancer through synthetic drugs. It is considered an effective treatment method for fighting cancerous cells that have been already spread to other parts of the body and that cannot be treated with any other traditional method[12]. Similar to radiation therapy, this therapy affect normal cells and cause the same unpleasant side effects.

Hormone therapy is one of the most recent treatments that involve anything that deals with manipulating the body's hormones to treat the cancer. These treatments can include administering hormones, drugs, or gene sequencing to produce a specific type of hormone or in the worst scenario the removal of hormone glands.

Immunotherapy is another recent treatment that also manipulates the normal functions of the body, but in this case the immune system response is the altered function. Patients are treated with medication that stimulates the immune system and active cells that can recognized and attack cancerous cells. Thanks to new technology available now, it is possible to develop many compounds of biological origin that are used to elicit an immune response such as Interferons, Interleukin 2 (IL2) and Monoclonal antibodies[11].

Gene therapy encompasses a wide range of treatment types that all use the same base, which is to modify cancer cells at the molecular level and replace a missing or bad gene with a healthy one.

Vaccination is the administration of antigen material or DNA sequencing, biological response modifiers, among others to stimulate and individual’s immune system. This method work by stimulating the immune response or helping the immune system ability to fight and destroy cancer cells. There are two broad cancer vaccines. Preventive vaccines are intended to prevent cancer from developing. Therapeutic vaccine to treat an existing cancer by strengthening the natural immune systems defenses against cancer.

Classification of Cancer Vaccine

The idea behind cancer therapy vaccine is that tumor cells or antigens stimulate the host immune system to produce special cells that kill cancer cells and prevent it is development [13]. Within this end, a variety of epitopes and cancer vaccine delivery method and the use of adjuvant’s are under study. In this section we are going to review some of the approaches for the cancer vaccines development that are currently in clinical trials.

1. Tumor antigen-based cancer vaccine uses tumor-specific antigens to stimulate an immune response. The rationale behind this vaccine is that the immune system will recognize the same
antigen on a tumor cell and be armed to mount a strong immune response against the cancer cell that could result in the destruction of it. Antigens can be proteins or segment of the peptide sequence. Once injected the antigen-based cancer vaccine the immune system will respond to the foreign antigen producing antibodies or cytotoxic T lymphocytes, called Killer T cells. The immune system can be boosted using only one antigen. However, has been show that using more than one antigen to stimulate the immune response could potentially increase the immune efficiency to attack and recognize cancer cells [14]. Ultimately, antigens can be genetically modified in order to make them more easily recognized by the immune system and get a stronger immune response.

1.1 *Tumor antigen-DNA vaccine* addresses DNA sequencing that encodes a tumor antigen into the antigen-presenting cells’ (APCs) nucleic acid sequence. The tumor antigen will produce in the cell and present on the cell surface as MHC-antigen complex which activates cytotoxic T cells. Now, there are large numbers of T- cells activated that can recognize and destroy cancer cells with the same antigen on the cancer cell surface[15].

1.2 *Tumor antigen-synthetic Peptide vaccine*: Consist in an introduction of a synthetic peptide-antigen into a normal cell to mount a strong immune response, much like the antigen-DNA vaccine. Mixes of synthetic peptide have shown success in clinical trials[16]. Synthetic peptide vaccine offers a number of advantages over other types of cancer vaccines. The production of peptides on a large scale can be considered easy and inexpensive. Regulatory approval is not hard because it is easy to characterize and analyze for purity [16]. Shortcoming of this type of vaccine could include weak immunogenicity of simple peptides, and the requirement that immune systems have the appropriate leukocyte antigens[15]. This vaccine is an advantage for undeveloped countries because it can be stored freeze-dried at ambient temperature, which facilitates storage, transport, and dissemination. Last but not less significant, there is no risk of deleterious sequence which could potentially promote either transformation or autoimmunity.

1.3 *Tumor antigen-carbohydrate vaccine* is a new prototype under study to be used as a preventive cancer vaccine for carcinoma at early stage of transformation. Thompsen-Freidenreich, glycopeptides found in carcinoma at early stage of transformation but not in normal cells, is used to induce an antibody response against carcinoma cells. Previous results from the University of California suggest that on effective cytotoxic T-cell receptors are capable of recognizing this tumor-associated carbohydrate antigen in a conventional class I MHC complex, holding a promise to be an efficient vaccine again carcinoma cancer cells[17].

1.4 *Exosome- based cancer vaccine*: Exosomes are small membranous vesicles capable of degrading various types of ribonucleic acid (RNA) molecules that are originated from endosomes. They are developed from the membrane of subcellular multi-vesicular bodies, bodies, fused with the plasma membrane, and are released extracellularly. Theses molecule refuse with the neighborhood cells' membranes. They are composed of different types of cytosolic molecules and membrane proteins. Furthermore, they have a dual function in which they represent a vehicle for removing unneeded cellular proteins and are a pathway for tracking proteins between cells[18]. Native tumor cells constitutively secrete tumor exosomes. Scientists believe that exosomes bring tumor antigens to dendritic cells and, through action of surface heat
shock protein (HSP) accelerate self-internalization in dendritic cells[17]. This was shown through the incubation of dendritic cells with tumor exosomes in vitro and in vivo in mouse models. The result in both scenarios was the activation of specific cytotoxic T-lymphocytes [18].

1.5 Tumor heat shock protein- based cancer vaccine There is evidence which clearly shows that HSP, such as GP69 in HSP70 isolated from tumor cells, can induce a specific antitumor immune response. Campoli and others showed that HSPs are able to bind the antigenic peptides developed in tumor cells, to be actively taken up by receptors in DCs by a process that mediates endocytosis and induces DC maturation through the interaction with DC Toll-like receptors. Once HSP is internalized, the antigenic peptides are released from HSP, and enter to antigen processing and cross-presentation process, finally forming a complex with MHC I on the surface of an antigen-presenting cell [19].

Previous data show that there are many advantage of HSP vaccine. The main advantage relies on the fact that HSPs contain many, if not all, tumor antigenic peptides, which enables them to induce a polyclonal antitumor immune response, bring antigens to DC and induce their maturation [19,20]. Many pre-clinical trials confirmed in vivo provide a strong immunogenicity of HSP70 or GP69 vaccines. They have sufficient data to prove the efficiency of this vaccine in the induction of prophylactic and therapeutic antitumor immune system responses in many pre-clinical trials [19]. The principal drawbacks of HSP vaccine is the time consuming isolation process of peptide-HSP complexes as well as unknown antigenic profile [19].

2. Monoclonal antibodies-based cancer vaccines are derived from identical immune cells that are all clones of a unique parent cells[21]. They present monovalent affinity—they bind to the same epitope-.

2.1 Anti-idiotype vaccine. Each B-lymphocyte generates only one kind of antibody. The unique part of each type of antibody is called an idioype as well as the variable region. Antibodies are generated when the immune system responds to an antigen. However, sometimes the immune system makes antibodies against antibodies. Campoli and others demonstrated that these antibodies against antibodies are important to keep the immune system under control. The anti-idiotype antibodies vaccine uses the variable region of B-lymphocyte as tumor-antigen to initiate an immune response against the variable region the antibody. Once introducing into the cell these antibodies bind tightly to the tumor-antigen. In addition, the anti-idiotype are recognized by other antibodies, creating a network and magnifying of the immune system response again to a specific cancer cell[19].

In vitro experiments of anty-idiotype have been conducted in animal tumor models and have shown that anti-idiotypic immune response can destroy a number of different tumor cells, including myeloma[22]. Scientists believe that because of the polyclonal nature of anti-idiotypic vaccine the immune response strongly reduces the immune-resistance of myeloma cells. Therefore, anti-idiotypic vaccines represent a promising immunotherapeutic antitumor strategy[23]. Drawbacks include their weak immunogenicity (idiotype is a self-protein).
However, previous studies show that conjugation of an idiotype-antigen with an immunogenic adjuvant can mount a strong efficient immune response[19].

3. Cell-based immunotherapy: The next type of vaccines involves the stimulation of the immune system against cancer cells by the injection of the entire cells into the patient. Up to now we have discussed the stimulation of the immune system by the introduction of molecules into the cell to generate a strong anti-cancer cell response.

3.1 Tumor cell-based vaccines are made up of whole tumor cells, taken either from the patient (autologous) or from a different patient (alloogenic). These cells are attenuated or killed before injection into the patient, which does not interfere with the antigen presentation at the tumor cell surface. Once injected into the patient, they stimulate the immune system to recognize the existing tumor and mount an immune response. Then the immune system will seek out through the whole patient-system and attack any other cells with these antigens that are still in the body[19]. Tumor cell vaccines have the advantage of having all the relevant tumor antigens needed by the immune system to mount an effective antitumor response. Another advantage of tumor cell-based vaccine is allowing immunization/treatment without knowing the specific antigens present in a particular tumor cells.

Tumor cell vaccines have been shown to be effective in mounting an immune response in most transplantable tumor mouse-model but less so in spontaneous cancer models that closely recreate human tumors development[23]. Scientists attribute this effect to the fact that the immune system's inherent tolerance to many tumor cells because they are some protein in tumor cells that are also present in normal cells[24]. It has been shown that such vaccine can be made more immunogenetic by genetic transformation of tumor cells to express co-stimulatory molecules. Previous studies have shown that vaccination with genetically engineered tumor cells modified to express granulocyte macrophage-colony stimulating factor (GM-CSF) have the potential to amplify immune responses by improved tumor antigen through increased dendritic cell and macrophage recruitment[25]. This modification enabling the generation of melanoma-specific T-helper cells and killer T cells, restricted Natural Killer cells and antibodies for successful induction of tumor rejection[26]. This immunization strategy is on phase I clinical trials in patient with secondary melanoma. So far the strategy has shown consistent induction of both cellular and humoral anti-tumor responses, thus allowing tumor destruction without noteworthy toxicities[19].

3.1.1 Tumor autologous cell-based vaccine; Autologous means “related to self”. These vaccines are made from attenuated tumor cells taken from the same organism in which they will later be delivered. To create a new and unique vaccine for each patient can be expensive. It is known that cancer cells have a high rate of mutation, which means an autologous tumor vaccine eventually will become less effective at the same rate that the cell mutates[20]. Furthermore, depending on the surgery and the tumor size is the amount of viable cell to make a vaccine. Sometimes after surgery there are not enough cells to make the vaccine and/or there not enough cell to re-treat if the cancer comes back[19].

3.1.2 Tumor alloegenic cell-based vaccine; Allogenic means “genetically different but it comes from the same species”. These vaccines are made from attenuated tumor cells taken from
someone other than the organism being treated. Generally these vaccines are made by shuttling tumor cell which were removed from several different organisms but belong to the same species. Allogenic vaccines have the advantage of being a treatment for more than one specific organism. Shortcomings include weak immunogenicity[20]. One can try to overcome this by using one or more adjuvant substances to stimulate the immune system.

3.2 Dendritic cell-based vaccine; Dendritic cells (DC) are specialized antigen presenting cells. Such cells help the immune system to recognize cancer cells[12]. These cells can break down cancer cells into small pieces which can include antigen. Later on, these antigens can be presented to T- cells. This helps the immune system to recognize cancer cells and attack them. Dendritic cells have to be individually created for each patient’s, autologous vaccine[20].

Osada and others have been able to differentiate dendritic cells for the development of DC vaccine. This process involves a complex and expensive process. Cells that differentiate into dendritic cells—for example monocytes- are isolated from the patient blood. These cells are cultivated in a medium and growth factor that stimulate the differentiation into dendritic cells. This process allows getting more autologous dendritic cells from the patient than if just isolate DC direct from the patient. Then these cells are either exposed to cancer cells or cancer antigen, with engineering modified to expresses the cancer cell antigen or fuse with tumor cells. Before injection unto a patient, the complex of DC-antigen or DC-tumor must undergo to a maturation process. This process is required for improving the ability to activate T-cells [28].

The potential of DC-based vaccines to induce an antigen-specific immune response has already been shown in many preclinical animal models and clinical trials. DC vaccine seems to be promising in terms of efficacy. There are many drawback still to overcome, such as the need to define a standardized protocol and to minimize time and cost production[29]. Scientists argue that choosing the best DC population from many DC cell subsets with distinct properties is still a challenge because DC subset has its unique capacity of activating either T-helper 1, T-helper 2 or T-helper 17 cells[27].

3.3 T cell-based vaccine; Recently different techniques have been develop for T cell- vaccine The first strategy, allogeneic lymphocyte therapy consists of taking the T cells from different persons and infusing them into the patient. The logic behind this process is that MHC antigen differences, together with the tumor antigens, sets up an immune response in which the T cells from the donor destroy the host’s tumor (graft versus tumor reaction)[30]. A second strategy describes by Armstrong and others involve manipulation of cytotoxic T cells to become able to recognize by themselves tumor cells, and become activated. In this approach the point is to bypass the involvement of MHC class I on tumor cells and antigen-presenting cells[31]. To meet this purpose a chimeric molecule was created, consisting of an antibody domain and a T-cell receptor (TCR) domain. As expected, the antibody domain recognizes the specific tumor and the TCR domain activates T cells to combat the tumor and secrete proliferation signals in the tumor environment. This approach has been successful in treating ovarian tumors in pre-clinical trials in mice[30].

More recently a new approach consisting of autologous tumor-specific T- lymphocytes has been taken into consideration. T-cells are isolated from the patient’s peripheral blood, tumor or tumor...
infiltrated lymph node. Then theses cell are activated and multiplied ex vivo in the presence of specific T-cell epitopes, and then returned to the patient system [32]. This approach offers a better immunotherapeutic option for patients with widespread disease and high mass of tumor tissue[33]. Currently, there are still many drawbacks that have to be overcome before this vaccine is developed to its maximum potential. The identity of tumor antigens used in the ex vivo activation of antitumor T-cells needs to be known and the process of antigen identification is difficult, time consuming, and expensive [32]. Another disadvantage is the ability of transferred lymphocytes of tumor infiltration and their shorter life span. However, previous studies have shown that the shorter survival of T-lymphocytes can be partially improved by adding Interleukin 2[23].

3.4 NK cell-based therapy: The rationale behind this process is the same as allogeneic lymphocyte therapy. Allogeneic Natural Killer (NK) cell therapy consists of taking the NK cells from a different person and infusing into the patient. In previous clinical trials the donor and hosts were mismatched for killer cell inhibitory receptors in carcinoma patients. NK cells from a donor produce a graft-versus-tumor response against the cancer cell, the same immune response obtained from allogeneic lymphocyte therapy[34].

4.1 DNA Vaccine: DNA vaccine has been developed with the purpose to provide a way to supply a steady antigen and keep the immune response going. The problem with the previous vaccines discussed are the once they are injected into the body as a vaccine, they usually cause the desire immune response at first. However, over time they may become less effective. The principal reason is that the immune systems recognizes them, antigen or cell, as foreign molecules and quickly destroys them. Without any other stimulation, the immune system will returns to its normal state of activity[23].

DNA vaccines are based on bacterial plasmid that has been engineered to express the desired gene under a eukaryotic promoter element that is active in mammalian cells. This vector can be a shuttle vector in which the plasmid has an origin of replication for eukaryotic cells as well as prokaryotic. Other vectors are designed to have a homologous sequence with the mammalian genome allowing them to incorporate the target sequence in the mammalian genome. The last vector does not have a eukaryotic origin of replication and is considered less hazardous. The idea behind both vectors is the same. Firstly, the antigen encoded is produced in the mammalian cell. Once the antigen is properly transcribed and folded it is recognized by either the humoral immune system or the innate immune system, creating a network respond against cells presenting the encode antigen[23].

The major advantages of DNA vaccines are the easy, cheap and convenient cost of production and purification. They do not require special handling or storage conditions. They mount immune responses only to the encoded antigens. They can both elicit innate and/or humoral immune responses. Recently the plasmid DNA-based vaccine has proven safe, and there are now several treatments using this type of vaccine in late-stage clinical testing[35]. So far, they appear to hold a promise for cancer therapy treatment. The only drawback until now if the specific integration of antigen-sequence into the genome interrupts an important or essential genome of the host cell, this potentially can foster malignancy [23].
Some scientists are encouraging the use of RNA vaccines instead of DNA vaccines. They argue that RNA vaccines are more safety to the host cell because can avoid the integration problem and the RNA can be submitted to degradation in a safe and economical process using RNAses[36]. However, RNA-based vaccines will not be able to supply steady antigens and keep the immune response on going as expected with DNA vaccines because RNAses is also present in body fluids and so they are not presenting any way to protect or delivery the RNA vaccine once injected.

Improving Efficacy and Immunogenicity of Cancer Vaccines

Vaccines have been successfully directed against a wide variety of tumors in mice-animal models, almost exclusively by driving immune responses against antigen-specific protocol. However, the same results have not been obtained in large animal models, such as pigs and humans. The reason for this failure is that it has not been able to induce a potent immune response in humans. Mojca Frank and others establish that there are three basic requirements for developing an effective immunotherapy cancer vaccine. The first main is to establish that the vaccine be developed with enough high-affinity tumor specific lymphocytes because, as discuses previously, tumors actively avoid the immune response by using a variety of mechanisms which can include the secretion of cytokines. Second, the tumor-specific lymphocytes must have to pass through cell-cell barrier in order to infiltrate into the tumor cell. Lastly, the infiltrated lymphocytes must effectively kill the tumor cells [37]. Even when tumor cells have developed strategies to evade antitumor response, there are some strategies to overcome this drawback and develop an effective cancer vaccine therapy. Some of the approaches that are on clinical trial to date to overcome those drawbacks are presented in this section, which includes a variety of tumor antigen delivery methods in combination with adjuvants, enhancing the microenvironment of tumor cells, among others.

Enhancing DNA sequence: From previous studies it is known that maximizing the expression of the antigen is critical to the specific induction of the immune responses. For the delivery of DNA vaccine it is recommended that the antigen-DNA sequence be under the control of a strong viral promoter, such as CMV-intA, or better than that be under the control of an endogenous eukaryotic promoter[38]. It is important to take into consideration the nucleoside amino acid sequence used to induce DNA expression using the optimal codon sequence taking in consideration amino acid sequence in abundance.

One approach have been developed using cytosine-phosphate-guanine (CpG) unmethylated which is present on bacterial DNA. This sequence has the function to stimulate the innate immune in eukaryotic organisms, creating an inflammatory response for triggering the adaptive immune response[39]. Preclinical studies showed that the addition of CpG motif as adjuvants in the patient plasmid can significantly increase the induction of pro-inflammatory secretion of cytokines, such as Interlukine-12 [40]. Previous data show that CpGs are recognized by TLR9. Toll-like receptor (TLR) plays a fundamental role in pathogen recognition and activation of innate immunity. TLR9 is a receptor found on antigen presenting cells that plays a role in the induction of helping cytotoxic T-lymphocytes in their differentiation process[41,42].

Microenvironment Enhancement: There is an immense array of molecules that can be delivered into the cell to modulate immune responses. These molecules include chemokines, protein
secreted by cells with the ability to induce directed chemotaxis, to attract antigen presenting cells and stimulate the immune system[43]. Microenvironment improving during the vaccination process has been shown to improve significantly the low immunogenicity of cancer vaccines[44].

**Vaccine Delivery Enhance:** The immune response has been enhanced by the improving of the delivery vaccine-technique. Progress has been developed in improved techniques for encapsulating the material to be delivery in DNA vaccines[45]. Plasmid made of liposome, polymers, and microparticles was set up for delivery of DNA vaccines. It had been shown that they have the potential to elicit a strong immune response in comparison to those elicited by simple intramuscular plasmid DNA[46]. This strategy has not been successful in animal models yet.

Epitope, known as an antigenic determinant is the part on an antigen that is recognized by the immune system cells, such as antibodies, B-lymphocytes and T-lymphocytes, among others. A better understanding of the epitope role on the immune system is leading to a new strategy to induce stronger immune responses. The scientific community is working on a process termed epitope enhancement. They are trying to sequence cancer epitopes more immunogenic, as expected not all sequences are optimal for antigenic recognition[47]. The initial modification of epitopes is based on modifying the epitope sequence to increase the affinity of the epitope peptide for the MHC molecule[48]. Scientists believe that this strategy can significantly increase the immunogenicity of the vaccine and convert a subdominant epitope into a dominant epitope[49], and in this way improve the delivery of cancer vaccine.

Plasmid design, together with the technology of gene manipulation allows gene optimization. Currently, the variable regions of the heavy (VH) and light chain (VL) of the immunoglobulin have been modified, to create an immune response specific to a particular tumor. Researchers also have been working cloning the VH and VL to create a shuttle library in order to identify a novel B-cell using only single chain of the variable fragment (scFv) format, encoding a single polypeptide consisting of VH and VL genes linked together by a short amino acid linker. This small structure facilitates the infiltration of modified-B immunoglobulin into the clumps of cancer cells, incrementing in this way the immunogenicity of the vaccine [50].

Another approach consists of increasing the immune efficiency of a vaccine using a fusion protein encoding to a prion inflammatory chemokine moiety that facilitates targeting of the antigen protein cells (APC) with the antigen joined to it. The efficiency of this model was shown by Biragun and colleagues in article entitled “Models for Lymphoma” [51].

**Route of administration:** It appears that the immunogenicity of the cancer vaccine depends on the delivery method used for immunization. Some of the methods used for the administration of the cancer vaccines are:

**Biolistic particle gun delivery:** This is a device using to introduce vectors or another micro particle with genetic information into the cell using a high pressured instrument. This system has been developed for gene and/ or particles transfer into various mammalian tissues. A broad range of somatic cell types has been successfully transfected in *ex vivo* and *in vitro* systems in suspension cells as well as adherent culture cells[51]. So far, the biolistic device enables delivery of DNA directly to transfect keratinocytes and epidermal Langerhans cells[52]. It have
been shown that this delivery system mounts a greater cytotoxic cell response as well as to require a lesser amount of vaccination delivery process to achieve cancer/tumor immunity[53]. It has been shown that the use of gun delivery for gene delivery can be improved using non-coating plasmid DNA because it induced a stronger cytotoxic T lymphocyte effect[54]. This effect is still on pre-clinical trials.

Electroporation (EP) is consider a promising strategy because in primates it increases the level and the breadth of response[55]. This overcomes the drawback of traditional method delivery in which the translating effectiveness of vaccination from preclinical trials in rodents to large animals (including human subject) was less efficient[56]. Electroporation causes a temporary membrane disintegration of the cell membrane which in turn causes this system itself to act as an adjuvant, enhancing the danger signals that becomes detectable by the immune system[57]. In other words, EP delivery significantly enhances cellular uptake of delivery vaccine.

Tatto device. This is a newly developed intradermal physical delivery for DNA injection to skin cells. The device has a cartridge of perforating needles that oscillate at a constant high frequency, leading to DNA be transferred to skin-associated cells[23]. This approach seems to decrease the time required for the induction of immune responses and protective immunity. Gene expression after DNA tattooing has been shown to be higher than that after gene gun delivery and intramuscular injection[58]. In addition, despite the fact that with this method only lower doses of DNA can be delivered which lead to decreased gene expression, DNA delivery by tattoo induces higher antigen-specific cellular as well as humoral immune responses than by intramuscular DNA injection [23]. The delivery of DNA by this system in the absence of adjuvants elicited a much stronger and more rapid humoral and innate responses than intramuscular needle delivery vaccine together with molecular adjuvants [58]

Ultrasound (US) is being utilized to transiently disrupt cell membrane and enable the incorporation of protein sequence into cells.[59] To enhance the protein delivery by this method a combination of therapeutic ultrasound and microbubble echo have been used a high wave at high wave pressure[60]. By this method DNA is effectively transferred into the cytosol cell. This approach already has been applied to deliver proteins into the cells, but has not yet been proved to deliver antigen into DCs for cancer therapy. In vivo and in vitro studies have shown that this system of ultrasound can aid in the colon carcinoma cell in mouse models. [59].This system needs to be studied in large animal models.

Break the Immunosuppressive Networks. Tumor microenvironment is characterized by immune suppression of the host cells which it is also a barrier to immune therapy. This tumor microenvironment is set up through the activity of granulocyte precursor cell, lymphoid, and through the production of immune-suppressive factor[29] One approach to break the tumor micro-environment is the used of myeloid-derived suppressor cells (MDSCs). They are macrophages with the potential to infiltrate through the barrier of tumor cells and can exert a strong anti-inflammatory effect. These kinds of macrophages are abundant in some tumors developed in humans as well as in mice. Another cell that can be potentially used to break the Immunosuppressive networks is the regulatory T cell (Tregs) which can heavily infiltrate into many tumors[61].

Briefly resuming, an effective cancer vaccine needs to overcome some tumor cancer cell responses. As previously discussed tumor cells can down-regulate expression of MHC and
antigen presenting cells and frequently secrete immune suppressive molecules to protect themselves from being attacked by the immune system[62]. In addition, cancer cells secrete soluble factors to the environment, such as VEGF, IL-10, and TGF-B that prevent the maturation, differentiation, and activity of antigen presenting cell as well as dendritic cells[63]. This is another factor in the cancer microenvironment needed to be overcome in order to increase the cancer vaccine therapy. There is a group of scientist that believe that the DNA vaccination can overcome these shortcomings suppressing the progression of already established tumors by targeting the secreted factors in the tumor microenvironment by the cancer cells and then reversing the immunological attenuation by improving the DNA vaccine platform[64].

Conclusion

The Centers for Disease Control and Prevention indicates that cancer is the second leading cause of death in the U.S.A and is considered one of the most devastating diseases worldwide[1]. A number of different types of cancer treatment therapies have been used to treat cancer developed cells. However, none of these traditional treatments stood out as potential anti-cancer therapy[65]. As a result, scientists are still working on developing an effective methods with high specificity and immunogenicity against cancer cells. Cancer vaccines represent a new approach to treat this devastating condition with high specificity and immunogenicity.

Since 1893, when William Coley reported the regression of soft-tissue sarcomas from patients with acute bacterial infections[8], scientists have tried to manipulate the immune system to generate cancer vaccine. The field of using active immune therapy has achieved substantial progress since the days of Coley. Improvements in the technology, progress in the understanding of the cellular pathways in the regulation of immune activation, control and tolerance, as well as a deeper understanding of tumor biology itself have resulted in the development of a variety of promising tumor vaccine prototypes[65]. Furthermore, the number of cancer vaccines under development in academic research laboratories as well as in the pharmaceutical and biotechnology industries contributes to the enhancing of cancer vaccines[67]. As a result, cancer vaccine is no longer a future approach; in essence the development of cancer vaccine is in our hands. However, the development of a universal cancer vaccine to day seems to be unrealistic because there are so many types and causes of cancer[68].

A number of different kinds of therapies are already developed, and none of them has the potential to cure cancer completely. Certainly the development of cancer vaccines has been relatively slow. We should take into consideration all the barriers that the immunotherapy has to overcome to destroy cancer cells effectively or prevent its development. However, the pace is increased substantially because scientists are gaining deeper understanding of the immune system response to tumors. As a result, to date there are vaccines approved by the US FDA for both preventive vaccine and therapy vaccine. Furthermore, there are many clinical trials that are under study to examine vaccines as potential treatments for a wide variety of cancer types[68]. Cancer vaccines promise to have many advantages in comparison with traditional methods to treat cancer. The greatest advantage is the ability to target both surface and intracellular antigens through the activation of the cellular immune response and humoral immune response, as well as the greater duration response, which avoid the need of long-term and multiple immune treatments[69].
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